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Airways of allergic rhinitics are 'primed' to repeated allergen inhalation challenge

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Summary

The hypothesis that repeated exposure to a specific allergen will further increase bronchial responsiveness to that allergen is supported by indirect evidence. However, it has not been tested as intensely in the laboratory setting, and in some cases, conflicting results are presented. In order to test the hypothesis in the atopic subjects, allergen inhalation challenge tests were performed in 29 house dust mite (*Dermatophagoides pteronyssinus*) sensitive subjects with allergic rhinitis. Nine subjects displayed early asthmatic responses (EARs) to the first challenge (Group I). Twenty subjects with no significant airway response were submitted to the second challenge 24 h later. Thirteen subjects showed EARs (Group II) and two of these showed late asthmatic responses (LARs) as well. In Group II, there were significant changes between the first and second challenge in post-allergen early phase FEV_1 (88·1 ± 4·2 vs 71·7 ± 4·2% baseline, $P < 0{\cdot}05$) and in post-allergen late phase FEV_1 (93·1 ± 3·4 vs 86·6 ± 7·8, $P < 0{\cdot}05$). After the second challenge, PD20 (provocative dose of methacholine required to produce a 20% fall in FEV_1) decreased significantly from the baseline values. When challenged separately with twofold dose of allergen, only three and one of the Group II showed EAR and LAR respectively. PD20 did not change significantly after this challenge. These results indicated that two repeated exposure to allergen dose, which is not enough to cause significant airway responses at a time, may provoke asthmatic airway responses in the subjects with allergic rhinitis and that this effect of priming is not attributed to the cumulative dose but to the consequent effect of repeated allergen exposure.

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Introduction

Airway hyperresponsiveness is a characteristic finding in the patients with asthma. The underlying mechanism in its development is presumed to be an airway inflammation [1]. This increase in airway responsiveness is not only important to the pathogenesis of asthma, but also further enhanced by antigenic exposure in atopic subjects. Thus, in sensitized subjects with asthma, laboratory or natural exposure of antigens leads to an increase in non-specific bronchial responsiveness (NSBR) measured by sensitivity to methacholine or histamine [2,3]. Cockcroft [4] proposed that repeated exposure to a specific allergen will further increase bronchial responsiveness to that allergen.

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Although this hypothesis is supported by the indirect evidences from studies of repeated allergen exposure [3,5] and from studies of allergen avoidance [6], it has been tested less completely in the laboratory setting and, in some cases, conflicting data are presented [7,8,9].

Nonetheless, the hypothesis is plausible because previous antigenic exposure could probably induce inflammation of the airways and eventually hyperresponsiveness to the specific antigen as well as non-specific stimuli. To test the above hypothesis in the patients with asthma by repeated allergen challenge has some limitations. Allergen challenge can provoke severe airway obstructions, especially in the late phase. They will require medications which would then interfere with the interpretation of the airway response to further challenge. Since the airway narrowing provoked by allergen challenge can last 36 h or longer, this would preclude further challenge

Table 1. Characteristics and allergen bronchoprovocation data of Group I subjects

Subject No.	Sex (M/F)	Age (year)	Height (cm)	Weight (kg)	Baseline PD20*	Allergen bronchoprovocation: Baseline FEV_1†	Allergen bronchoprovocation: Early phase FEV_1‡	Allergen bronchoprovocation: Late phase FEV_1‡
1	M	6·6	127	30	1·75	87·6	41·0	97·4
2	F	10·7	146	37	1·69	102·5	66·7	72·5
3	F	7·9	126	26	1·45	103·5	73·7	62·9
4	M	12·7	147	38	2·48	103·6	78·6	85·7
5	F	9·1	134	27	1·90	101·9	36·1	86·6
6	M	12·0	156	52	2·71	94·5	78·7	92·0
7	M	7·9	129	29	2·49	104·3	71·8	87·2
8	M	7·6	122	23	1·69	103·5	56·3	68·8
9	M	9·8	139	43	2·10	90·0	69·0	90·5
Mean	6M/	9·4	136·2	34·1	1·96	99·0	66·0	82·6
SD	3F	2·1	11·5	9·1	0·53	6·5	12·6	11·7

* Provocation dose of methacholine which caused a fall in FEV_1 of 20% from the baseline. It is calculated as the cumulative breath units, with 1 breath unit equal to one inhalation of 1 mg/ml methacholine, and expressed as the log-transferred values; † % predicted for height [16a]; ‡ % baseline.

within a few days [10]. Furthermore, it is difficult to document the further decrease in airway function responding to the next challenge once the response to the first challenge is significant.

We chose to test this hypothesis in the patients with allergic rhinitis rather than asthma for the following reasons. Firstly, even though the patients with allergic rhinitis do not have overt asthma, airway responsiveness is often increased [11,12] and further enhanced after laboratory exposures to allergen [13,14]. Secondly, when challenged with allergen, they display airway reactions of a lesser degree than subjects with asthma, but they can exhibit asthmatic responses with increasing exposure dose [15]. Thirdly, they can manifest intense allergic reactions in the bronchi even without severe airway obstructions after allergen challenge [16].

Our objective in this study was to determine whether the airway response to a specific allergen and the consequent NSBR are altered by repeated allergen challenge. To accomplish this, we submitted allergic rhinitics with no significant airway response after the initial allergen challenge to repeated challenge with the same allergen, and airway response and consequent NSBR were assessed. Additionally, we were intending to determine whether the total dose of allergen exposed or the exposure pattern is important for these changes. Thus, we submitted the same subjects to 'double' dose allergen challenge 2 months later, and the data were compared with those after the repeated allergen challenge.

Materials and methods

Twenty-nine children (19 boys, 10 girls) aged between 6 and 15 years (mean age = 10·5 ± 2·7 years) with perennial allergic rhinitis were selected to take part in this study (Tables 1 and 2). They were symptomatic (sneezing, nasal stuffiness, rhinorrhea, and/or nasal itching) throughout the year (lasting for at least 1 year). None of the patients had a clinical history of asthma (absence of dyspnoea, chest tightness, or wheezing), physical examination as well as spirometry had been normal at the time of the clinic visits. All the patients had positive immediate skin reaction by the prick method to an extract of house dust mite (*Dermatophagoides pteronyssinus*).

They had been given nasal cromolyn sodium for several months, but it was stopped at least 2 weeks before the study. All subjects were taking no other medications at the time of the study, and were free of acute respiratory infections. All subjects provided statements of informed consent, and the study protocol was approved by the Hospital Ethics Committee.

Study design (Fig. 1)

After a preliminary screening visit (history, physical examination, skin tests, and bronchial methacholine challenge), patients were subjected to the first 'single' dose allergen bronchoprovocation during winter season of 1991. Those who showed early asthmatic response (EAR) and/or late asthmatic response (LAR) to the provocation

Table 2. Characteristics and allergen bronchoprovocation data of Group II and III subjects

						First allergen bronchoprovocation			Second allergen bronchoprovocation		
Subject no.	Sex (M/F)	Age (year)	Height (cm)	Weight (kg)	Baseline PD20*	Baseline FEV_1†	Early phase FEV_1‡	Late phase FEV_1‡	Baseline FEV_1†	Early phase FEV_1‡	Late phase FEV_1‡
Group II											
1	M	12·4	152	60	2·66	107·4	87·3	92·9	105·7	77·4	88·7
2	F	10·8	144	37	2·39	89·2	86·0	88·4	91·3	75·0	80·4
3	M	8·3	136	30	2·66	91·2	97·5	110·0	93·4	73·1	95·1
4	M	12·8	154	45	3·17	104·1	82·1	98·4	103·7	75·0	87·3
5	M	11·3	153	53	2·21	93·9	83·9	92·9	95·6	71·9	89·5
6	M	9·3	136	28	2·56	98·0	93·0	95·3	100·3	68·2	90·9
7	M	12·8	150	39	2·38	100·3	83·3	92·9	98·5	75·0	90·2
8	M	11·8	146	50	2·28	99·8	88·7	92·5	97·9	63·3	78·8
9	M	12·8	154	41	2·07	100·8	89·3	91·8	104·1	66·7	96·5
10	M	13·4	158	55	2·78	105·9	83·8	88·2	102·8	69·7	63·6
11	F	7·4	120	24	3·24	103·3	86·7	93·0	99·8	75·9	87·9
12	F	10·4	133	31	2·91	87·8	88·9	94·4	86·5	67·6	87·3
13	M	10·5	140	34	1·89	92·5	84·1	92·0	91·4	73·6	89·7
Mean	10M/	11·1	144·5	40·8	2·46	98·0	88·0§	93·1§	97·9	71·7	86·6
SD	3F	1·9	10·7	11·7	0·35	6·5	4·3	3·4	6·0	4·2	7·8
Group III											
1	M	8·9	145	37	2·81	88·4	90·1	100·0	86·8	89·3	94·6
2	F	8·2	136	29	2·85	107·7	91·1	96·7	105·3	86·4	88·6
3	F	14·9	164	40	2·77	101·3	92·3	91·5	98·2	85·9	95·2
4	F	12·6	148	45	2·93	89·5	97·8	93·7	86·6	98·9	94·4
5	M	11·3	140	37	2·74	104·4	91·5	89·4	102·2	86·9	95·7
6	M	14·8	172	60	3·09	92·8	98·8	99·4	94·2	96·4	98·8
7	M	14·3	167	52	2·89	104·8	98·7	97·4	104·8	98·7	96·7
Mean	4M/	12·1	153·1	44·3	2·84	98·4	95·6	95·7	96·9	92·2	94·9
SD	3F	2·7	14·3	8·8	0·14	6·0	3·8	3·9	7·9	5·6	3·1
Total											
Mean	14M/	11·4	147·5	42·0	2·58	98·1	90·7	94·6	97·6	78·9	89·5
SD	6F	3·2	12·3	10·6	0·38	6·8	5·4	3·8	6·6	11·0	7·6

* Provocation dose of methacholine which caused a fall in FEV_1 of 20% from the baseline. It is calculated as the cumulative breath units, with 1 breath unit equal to one inhalation of 1 mg/ml methacholine, and expressed as the log-transformed values; † % predicted for height (16a); ‡ % baseline; § $p < 0{\cdot}05$ compared with corresponding value of the second allergen bronchoprovocation in Group II by paired t-test.

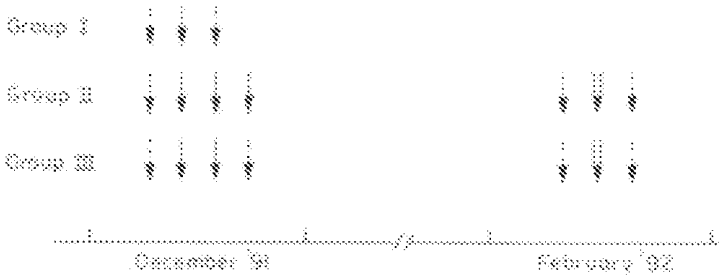

Fig. 1. Schematic flow chart of study design. The interval between each challenge test in one period was 1 day. The interval between the two periods was at least 2 months. I. Methacholine challenge test. II. 'Single' dose allergen challenge test. III. 'Double' dose allergen challenge test.

(Group I) returned next morning for the methacholine challenge test.

Those who showed neither EAR nor LAR (Group II & Group III) were subjected to the second 'single' dose allergen bronchoprovocation in the next morning, and a methacholine challenge test was performed on the following day. Two months later, after baseline measurement of methacholine sensitivity, these were subjected to the 'double' dose allergen bronchoprovocation, followed by methacholine challenge test 24 h later.

On each day of the study, subjects arrived at the laboratory at 08.00 hours and lung function was measured with a computerized spirometer (Microspiro-HI 298, Chest, Japan) after a 30 min rest. The study was continued only if the baseline FEV_1 before each test was 70% as predicted [16a]. The largest value of the triplicate FEV_1 at each time was used for the analysis. During the whole day, subjects stayed in the laboratory and did not take any medication or caffeine.

Methacholine inhalation test

Methacholine bronchial challenges were carried out by a modification of the method described by Chai *et al.* [17]. The concentrations (0·075, 0·15, 0·3, 0·625, 1·25, 2·5, 5, 10, 25, 50, 100, 150 mg/ml) of methacholine (Sigma Chemical, St Louis, MO, USA) were prepared with dilution in buffered saline (pH 7·4).

A Rosenthal-French (Laboratory for Applied Immunology, Baltimore, MD, USA) dosimeter, triggered by a solenoid valve set to remain open for 0·6 s, was used to deliver the aerosol generated from a DeVilbiss 646 nebulizer with pressurized air at 20 psi. Each subject inhaled five inspiratory capacity breaths of buffered saline and increasing concentrations of methacholine at 5-min intervals until the FEV_1 fell by more than 20% from baseline. The concentration of methacholine which caused a fall in FEV_1 of 20% (PC20) was obtained from the log concentration-per cent fall in FEV_1 curve by linear interpolation of the last two points. The results were expressed as the cumulative dose (PD20), with 1 breath unit of methacholine equal to one inhalation of 1 mg/ml methacholine [17].

Allergen challenge test

Allergen challenge tests were performed with a simple modification of the method described by Chai *et al.* [17]. The extracts of house dust mite (*D. pteronyssinus*) were obtained from Bencard, UK, and diluted with buffer phosphate. Serial alternative five- and twofold dilutions were prepared as described (10^{-5}, 2×10^{-5}, 10^{-4}, 2×10^{-4}, 10^{-3} w/v concentrations), and inhaled starting with 10^{-5} w/v after a control inhalation of buffer phosphate. The baseline values of each allergen test were FEV_1 values obtained after inhaling buffer solutions just before the allergen exposure.

Aerosols were generated by the similar manner as the methacholine challenge. For the 'single' dose allergen challenge, each subject inhaled five inspiratory capacity breaths of serial concentrations of allergen. For the 'double' dose allergen challenge, each subject inhaled ten breaths. Inhalations were continued at 15-min intervals until there is a 20% fall or more from baseline or the highest concentration of 10^{-3} w/v was administered. After the last concentration, FEV_1 was measured at hourly interval for 10 h. Response was expressed as FEV_1% baseline (FEV_1/baseline $FEV_1\times100$) measured at 15 min (early phase) and the minimal FEV_1% baseline between 3 and 10 h (late phase) after the last concentration of allergen. The EAR or LAR was defined when FEV_1% baseline of the early or late phase is below 80% or 85%, respectively.

Statistical analysis

All PD20 values were log-transferred before the analysis. Data are presented as mean ± 1 SD, except for PD20 as geometric mean and range of 1 SD. Differences between means for paired data were tested for significance following appropriate parametric or non-parametric statistical procedures. Comparison of values between the groups were performed using Wilcoxon rank sum test. In each case, statistical significance was accepted when $P<0·05$.

Results

Nine of 29 subjects showed EAR to the first 'single' dose allergen challenge. The subjects were designated as Group I. Of 20 non-responders to the first 'single' dose challenge, 13 subjects showed EAR to the second 'single' dose challenge, which was performed 24 h after the first

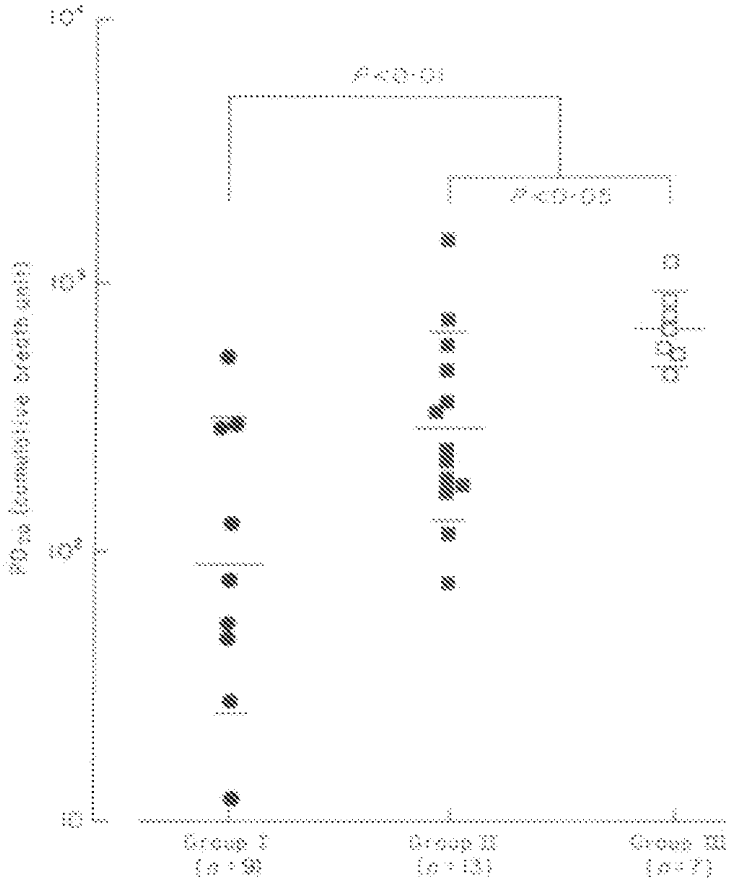

Fig. 2. Comparison of baseline methacholine responsiveness in each group of subjects. Data are expressed as provocation doses (PD20) of methacholine required to reduce FEV_1 by 20% and geometric means and 1 SD of each group are indicated with horizontal bars.

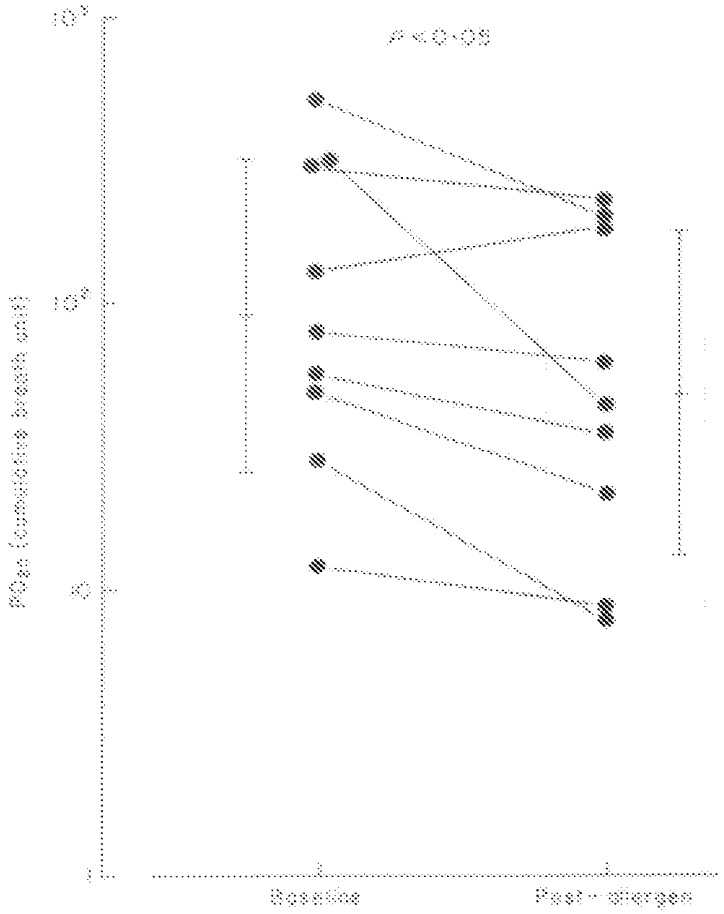

Fig. 3. Changes in methacholine responsiveness after allergen challenge in individual subjects of Group I. Data are expressed as provocation doses (PD20) of methacholine required to reduce FEV_1 by 20% and geometric means and 1 SD of each group are indicated with horizontal bars.

challenge, and the rest ($n = 7$) did not. The former subjects were designated as Group II and the latter as Group III.

Data of allergen challenge in the Group I is shown on Table 1. Three subjects showed not only EAR but also LAR. No significant difference was observed in skin-test data determined by weal size (not shown), and baseline FEV_1 expressed as % predicted for height between the Group I and the other groups combined. However, the subjects in the Group I were younger than those in the other groups (9·4 ± 2·1 years vs 11·4 ± 2·2, $P < 0.05$) and PD20 of methacholine were lower (geometric mean, range of 1 SD: 91·6, 26·9–312·5 vs 390·8, 177·1–862·6, $P < 0.01$) (Fig. 2). The three subjects with dual responses had even significantly ($P < 0.05$) lower PD20 than the rest of the Group I. After the allergen challenge, PD20 of methacholine decreased significantly from baseline as a group (47·4, 12·9–173·8 from 91·6, 26·9–312·5, $P < 0.05$ (Fig. 3).

Data of the first and second 'single' dose allergen challenge in the Groups II and III are shown on Table 2. None responded to the concentrations of less than 10^{-3} w/v in the second challenge, so all the subjects inhaled up to 10^{-3} w/v as in the first challenge. No significant difference was observed in skin test data (not shown), baseline FEV_1, and age between both groups, but baseline PD20 of methacholine in the Group II was significantly lower than that of the Group III (Fig. 2) (287·1, 128·7–640·6 vs 691·8, 496·3–964·5, $P < 0.05$). Two subjects of the Group II showed LAR as well to the second allergen challenge. There were changes between the first and second challenge in postallergen early phase FEV_1 (90·7 ± 5·4 vs 78·9 ± 11·0, % baseline) and in postallergen late phase FEV_1 (94·0 ± 3·8 vs 89·5 ± 7·6) in the two groups combined. To analyse each group separately, the changes were statistically significant in the early phase (88·1 ± 4·2 vs 71·7 ± 4·2, $P < 0.05$) and in the late phase (93·1 ± 3·4 vs 86·6 ± 7·8, $P < 0.05$) in the Group II, but not significant in the early or late phase in the Group III (Table 2). After the second challenge, PD20 of methacholine decreased significantly from baseline in both groups

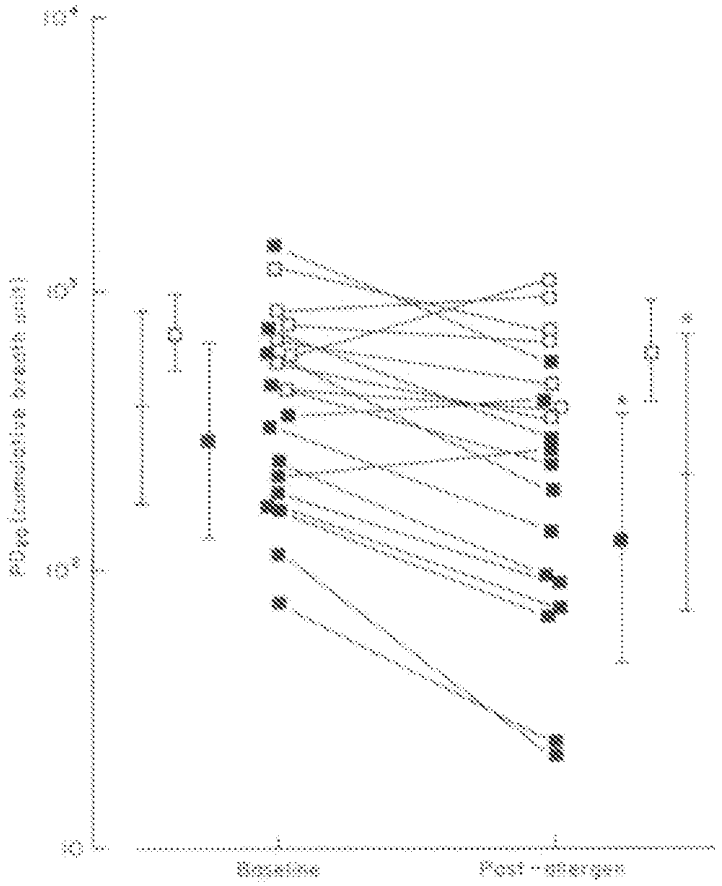

Fig. 4. Changes in methacholine responsiveness after the second allergen challenge in individual subjects of Group II (■) and Group III (○). Data are expressed as provocation doses (PD20) of methacholine required to reduce FEV_1 by 20%, and geometric means and 1 SD of each group or the groups combined are indicated with horizontal bars. *$P < 0{\cdot}01$ compared with the baseline by paired *t*-test.

combined (221·3, 71·4–685·7 vs 390·8, 177·1–862·6, $P < 0{\cdot}01$), but the changes were significant only in the Group II (128·5, 46·9–352·5 vs 287·1, 128·7–640·6, $P < 0{\cdot}01$) (Fig. 4).

In order to determine whether the changes provoked by the second challenge is due to cumulative dosage of two consecutive challenges or not, we performed another challenge in the subjects of Group II and III, at this time, 'double' dose allergen challenge. During the challenge process, all the subjects reached the concentration of 10^{-3} w/v, and only three subjects showed EAR and one showed LAR. The comparison of FEV_1 between after the second 'single' dose and after the 'double' dose allergen challenge is shown in Fig. 5. The mean level of FEV_1 as a group was significantly lower in the early phase after the second 'single' dose than after the 'double' dose (78·9 ± 11·0 vs 87·1 ± 6·7, $P < 0{\cdot}01$). However, when the data is analysed separately in each group, the difference was significant in the Group II(71·7 ± 4·2 vs 83·2 ± 4·1, $P < 0{\cdot}01$), whereas the difference was not significant in the Group III (92·2 ± 5·6 vs 94·5 ± 3·7, $P > 0{\cdot}1$) (Fig. 5a). There was no significant difference in the late phase FEV_1 between both challenges, when analysed in combination of both groups or separately in each group (Fig. 5b).

PD20 of methacholine measured one day before the 'double' dose challenge was used for the baseline values of changes in NSBR after this challenge. These baseline values were comparable to the initial baseline values. PD20 of methacholine after the 'double' dose challenge was not significantly different from the baseline values when analysed in combination or separately in each group (Fig. 6).

Discussion

We found that, at least sometimes, allergen exposure may lead to 'priming' with enhancement of the early or late airway response to subsequent exposure in the patients with allergic rhinitis. The results also indicated that the pattern of allergen exposure rather than the total dose may be important in the bronchial 'priming' to inhaled allergen and allergen-induced increase in NSBR.

Since allergic rhinitis is often associated with increased airway responsiveness [11,12] and has been suggested to be a risk factor for the development of asthma [11,18], some studies have tried to show abnormalities in pulmonary function pertaining to allergen exposure [13,14,19]. NSBR can be increased and seasonal bronchoconstriction may ensue during natural exposure to allergen in the patients with allergic rhinitis. Several studies [15,20,21] have shown that they may sustain a reduction in specific airway conductance or maximal expiratory flow rates after inhaling aerosolized pollen extracts. Although the dose of allergen that produced these changes was greater in rhinitics than asthmatic subjects, one study [15] showed considerable overlap of allergen sensitivity between the two groups. In the present study, we also noted that some subjects with allergic rhinitis exhibited the early airway response in the 'asthmatic' range to the first allergen challenge. This group was younger and had increased baseline NSBR compared with the groups lacking EAR. Recently, Muller *et al.* [22] reported that responsiveness of subjects with rhinitis to allergen had a closer correlation with methacholine responsiveness than was true in asthmatic subjects. An interesting point is that three of the Group I showed LAR as well. Muller *et al.* [22] reported that LARs are also seen in allergic rhinitis, although the incidence and severity were lower than those in allergic asthma.

The other subjects (Groups II and III) had no response in the early phase as well as late phase to the first allergen

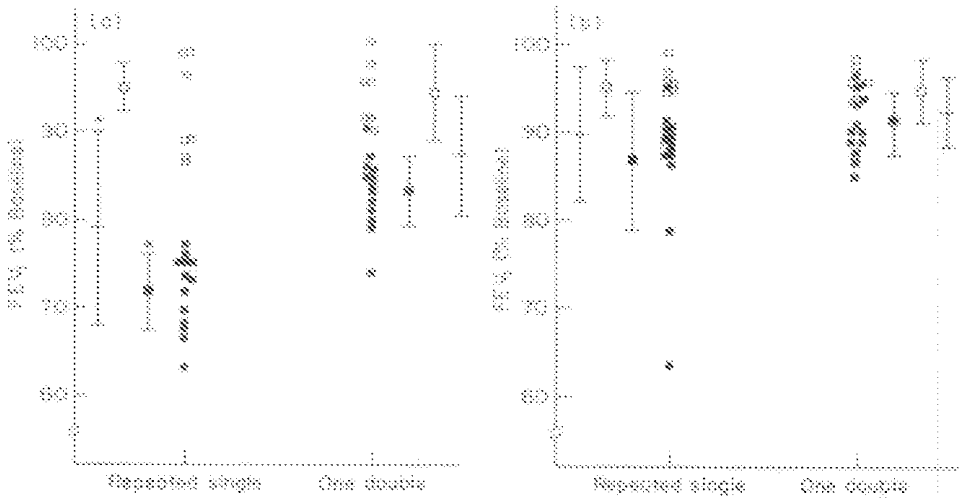

Fig. 8. Comparisons of FEV_1 in the early phase (a) and in the late phase (b) in individual subjects of Group II (■) and Group III (□) after inhaled allergen when the same dose of allergen was administered by either repeated single dose or one double dose. Data are expressed as percentages of baseline FEV_1 of each challenge, and means and 1 SD of each group or the groups combined are indicated with horizontal bars. $^{**}P < 0.01$ compared with one double dose challenge by paired *t*-test.

challenge, the dose of which was sufficient to provoke dual asthmatic response in most asthmatic patients [23]. But most of them (13/20) (Group II) exhibited the asthmatic responses to the second allergen challenge, which was performed 24 h later, when pulmonary function was recovered to the baseline level. Therefore it is rational to assume that the airway of allergic rhinitics may be 'primed' with sub-clinical dose of allergen. The 'priming' by the prior antigen challenge of the airway to the subsequent antigen exposure is similar to that identified in the nose. Connel [24], in his description of quantitative intranasal challenge with ragweed pollen, noted an increased nasal reactivity following repeated challenges in ragweed sensitive patients. However, there have been controversies as to the airway response to the repeated antigen challenge not only in human subjects, but also in animal models. Herxheimer [7] reported bronchial 'desensitization' to pollen in a limited number of patients using gradually increasing extract concentrations and durations of exposure. Kleeberger *et al.* [25] have shown that Bi-weekly antigen challenge causes a reduction in antigen-induced changes of lung resistance and compliance in sheep. Andrew *et al.* [26] also found that repeated exposures to antigen aerosol in immunized guinea-pigs resulted in a loss of antigen-induced bronchoconstriction. Rosenthal *et al.* [8], however, observed no regular trend toward either 'priming' or 'desensitization' in the serial bronchoprovocation in the subjects with asthma. Furthermore, there has been an increasing amount of literature suggesting that the repeated antigen challenge primes the airway response. Multiple intratracheal instillation of antigen-coated beads [27] or repeated antigen inhalation over 4 weeks [28] induced remarkable increases in airway inflammatory cells and responsiveness in primates. The same investigators [29] reported that multiple inhalations of antigen induced an increase in antigen-induced bronchoconstriction in the same model. Erjefält and Persson [30] described that two separate airway exposures to a low inflammatory dose of toluene diisocyanate increased about 10-fold the airway mucosal sensitivity to this agent in guinea-pigs. In a drug study on allergen-induced asthma in man, Cockcroft *et al.* [9] noted that some of the subjects who initially had an isolated EAR with no induced increases in NSBR developed definite increases in NSBR and equivocal LARs following the second or third allergen test. The conflicting results as described above may be due to differences in species, subjects tested, allergen dose, and interval administered. This study was done on patients with allergic rhinitis to ensure that some degree of allergic reaction would likely follow the challenge with large doses of allergen without the possibility of severe bronchial obstruction which might occur in individuals with asthma. Twenty-four hours were chosen as the time interval for repeated allergen challenge because it represents as the time period that reasonably reflects the entire spectrum of inflammatory response in the lung by allergen [31], which we assumed as the possible mechanism eliciting heightened response to further allergen challenge.

The mechanism by which the 'priming' of the airway by allergen exposure occurs is not clear but speculative. Bronchoalveolar lavage (BAL) data from human studies as well as animal studies suggest that airway inflammation with eosinophils and possibly neutrophils is important for the production of both LAR and increases in NSBR[32,33]. In contrast to the abundant data of airway cellular response in asthma, there are few studies performed in allergic rhinitis. Lam *et al.* [34] compared BAL in allergic rhinitis before and 10 min after inhalation challenge with antigen, but the time interval of BAL was too short for the inflammatory cells to be recruited.

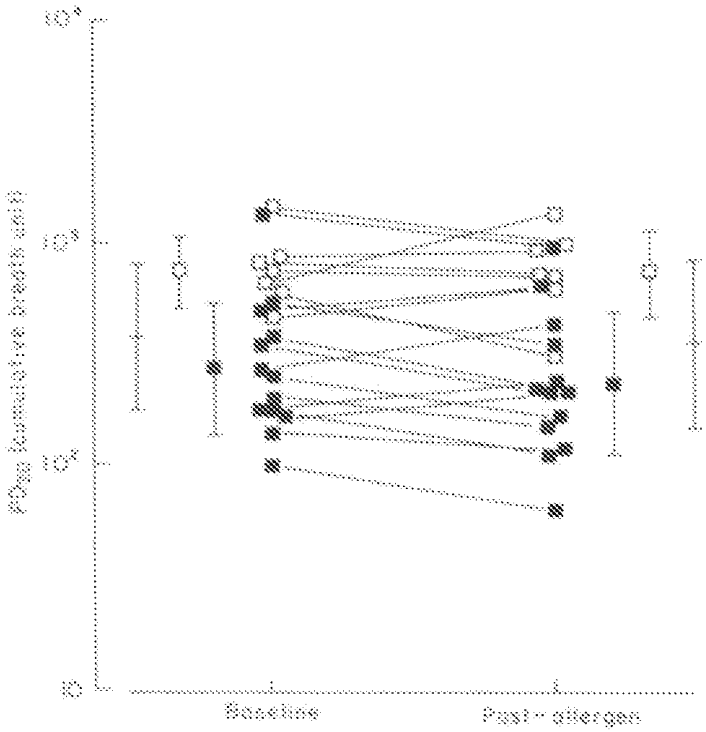

Fig. 6. Changes in methacholine responsiveness after one double dose allergen challenge in individual subjects of Group II (■) and Group III (□). Data are expressed as provocation doses (PD20) of methacholine required to reduce FEV_1 by 20%, and geometric means and 1 SD of each group or the groups combined are indicated with horizontal bars.

Boulet *et al.* [35] compared BAL data between during season and out of season in subjects with pollen-induced rhinitis, which showed no difference. However, we [36] have previously shown that significant number of neutrophils and/or eosinophils was recruited to the airway lumen 24 h after segmental allergen challenge in allergic rhinitics. In that study, we did not measure NSBR, but the airway reactions were minimal if any. This suggested that airway inflammation may follow allergen challenge in allergic rhinitis without preceding airway response. This is consistent with the data in asthmatics by Cartier *et al.* [37] and those in allergic rhinitics by Corren *et al.* [38]. Therefore it is presumable that airway inflammation may have existed after the first allergen challenge, although our cases in the present study did not show LAR to this challenge. We did not measure changes in NSBR in order to avoid affecting the result of the second allergen challenge test. Even if NSBR had not changed, allergen exposure might be a greater stimulus than non-specific agents to invoke the change of airway reactivity brought about by the first allergen challenge [39]. Consequently one may speculate that changes in smooth muscle responsiveness to mediator released from mast cells or other cells in the early phase or augmentation of inflammation in the late phase could possibly occur following the second allergen challenge. Another possibility is that the first allergen challenge may have resulted in infiltration of the bronchial mucosa by cells from the circulation that carry allergen-specific IgE, namely basophils. If that was the case, the number of target cells for the second allergen challenge may be increased leading to the release of larger amount of inflammatory mediators and induction of a stronger early and subsequently late reaction to the allergen.

The reason for the heightened response to the second allergen challenge may be attributed to the cumulative dose effect of allergen. For the double dose challenge, only three subjects showed EAR and one showed LAR. The mean magnitude of the early response was significantly lower than that of the second challenge. Furthermore, changes of NSBR were not significant between the baseline and 24 h after the double dose challenge. These findings suggested that the priming effect of the airway to further allergen exposure results from the pattern of exposure rather than the cumulative dose.

We used house dust mite (*Dermatophagoides pteronyssinus*), one of the most important perennial allergens all over the world. We admit that the degree of continual exposure to this allergen may have been changed during the present study. However, precautions were taken against this. Our study was performed between December and February, when indoor levels of the relevant allergen have been found to be the lowest and unchanged [39a]. We do not think that any other concomitant allergen exposure, such as animal danders or pollen, influenced the results of this study, because all the subjects had negative reactions to those allergens.

The possibility that the current results may be of clinical relevance remains intriguing. It is unlikely that airways are ever exposed to a dose of allergen as high as the dose used for this allergen challenge. These challenges, therefore, do not precisely mimic naturally occurring exposure. Nonetheless, the phenomenon observed in this study may have added advantages to investigation into the mechanisms involved in the pathogenesis of airway hyperreactivity in the atopic subjects. For example, the allergen content in the air might be too low to cause an asthmatic response but still enough to raise bronchial inflammation in the atopic subjects, and repeated allergen exposure can induce or enhance bronchial responses.

In conclusion, we have demonstrated in the subjects with allergic rhinitis that the airways can exhibit not only EAR but also LAR to allergen challenge, two repeated exposure to allergen dose, which is not enough to cause significant airway responses at a time, may provoke asthmatic airway responses and that this effect of priming is not attributed to the cumulative dose but to the consequent effect of repeated allergen exposure.

Although more data are needed, these results suggest that airways of allergic rhinitis can behave as those of allergic asthma according to the pattern of allergen exposure. Thus, avoidance of chronic exposure to allergens in allergic rhinitis is important in reducing not only nasal symptoms but also respiratory symptoms.

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